

=> fil reg; d que 16

FILE 'REGISTRY' ENTERED AT 15:09:21 ON 14 JUL 2000  
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STRUCTURE FILE UPDATES: 13 JUL 2000 HIGHEST RN 277295-36-0  
 DICTIONARY FILE UPDATES: 13 JUL 2000 HIGHEST RN 277295-36-0

TSCA INFORMATION NOW CURRENT THROUGH JANUARY 11, 2000

Please note that search-term pricing does apply when  
 conducting SmartSELECT searches.

Structure search limits have been increased. See HELP SLIMIT  
 for details.

*alpha chain*  
*Position 11 or 13 or 20 forced to change. Other positions may or may not change*  
*3 separate query segs combined into one search statement*

L6 1 SEA FILE=REGISTRY ABB=ON APDVQDCPEC[RKH]L[QRHK][ERHK]N[PRHK][F  
 RHK]FS[QRHK]PGAPIL|APDVQDCPEC[TRKH]L[RHK][ERHK]N[PRHK][FRHK]FS[  
 QRHK]PGAPIL|APDVQDCPEC[TRKH]L[QRHK][ERHK]N[PRHK][FRHK]FS[RHK]PG  
 APIL/SQSF - family search - conservative substitution allowed. See sheet attached  
 at end of this part of search.

=> d sqd 16

L6 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2000 ACS  
 RN 221650-43-7 REGISTRY - use Registry # to match citation to sequence  
 FS PROTEIN SEQUENCE

SQL 92

SEQ 1 APDVQDCPEC TLKKNKFFSK PGAPILQCMG CCFSTRAYPTP LRSKKTMLVQ  
 =====

51 KNVTSSESTCC VAKSYNRVTV MGGFKVENHT ACHCSTCYHH KS

HITS AT: 1-26

*Lys scanning mutagenesis?*

=> d que 17

*alpha chain* *Position 14 or 16 or 17 forced to change. Other*

L7 1 SEA FILE=REGISTRY ABB=ON APDVQDCPEC[TRKH]L[QRHK][RHK]N[PRHK][F  
 RHK]FS[QRHK]PGAPIL|APDVQDCPEC[TRKH]L[QRHK][ERHK]N[RHK][FRHK]FS[  
 QRHK]PGAPIL|APDVQDCPEC[TRKH]L[QRHK][ERHK]N[PRHK][RHK]FS[QRHK]PG  
 APIL/SQSF - family search

*positions may or may not change*

=> d sqd 17

L7 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2000 ACS

RN 221650-43-7 REGISTRY

FS PROTEIN SEQUENCE

SQL 92

SEQ 1 APDVQDCPEC TLKKNKFFSK PGAPILQCMG CCFSTRAYPTP LRSKKTMLVQ  
 =====

51 KNVTSSESTCC VAKSYNRVTV MGGFKVENHT ACHCSTCYHH KS

HITS AT: 1-26

=> d que 110

Searched by Barb O'Bryen, STIC 308-4291

*beta chain* substitution at positions 58, 63, 69

L10 3 SEA FILE=REGISTRY ABB=ON VCTYRDF[RHK]YRTV[ERHK]IPGCP[LRHK]HVAP  
YFSYPVA|VCTYRDF[IRHK]YRTV[RHK]IPGCP[LRHK]HVAPYFSYPVA|VCTYRDF[IR  
HK]YRTV[ERHK]IPGCP[RHK]HVAPYFSYPVA/SQSFP

*-family search*

*3 seqs  
combined in  
one search statement!*

=> d sqd 110 ~

L10 ANSWER 1 OF 3 REGISTRY COPYRIGHT 2000 ACS  
RN 221650-47-1 REGISTRY  
FS PROTEIN SEQUENCE  
SQL 118

SEQ 1 FCIPT EYTMH IERRE CAYCL TINTTICAGY CMTRDINGKL FLPKYALSQD  
51 VCTYRDFIYR TVRIPGCPHH VAPYFSYPVA LSCKCGKCNT DYSDCIHEAI  
=====

101 KTNCTKPKQK SYLVGFSV

HITS AT: 51-80

=> d 110 2-3 sqd

L10 ANSWER 2 OF 3 REGISTRY COPYRIGHT 2000 ACS  
RN 86179-24-0 REGISTRY  
FS PROTEIN SEQUENCE  
SQL 138

SEQ 1 MSAAVLLSVL FALACGQAAS FCIPT EYTMV VDRRE CAYCL TINTTICAGY  
51 CMTRDINGKL FLPKYALSQD VCTYRDFIYR TVEIPGCPHH VTPYFSFPVA  
=====

101 VSCKCGKCNT DNSDCIHEAV RTNYCTKPQS FYLGGSFV

HITS AT: 71-100

L10 ANSWER 3 OF 3 REGISTRY COPYRIGHT 2000 ACS  
RN 86179-23-9 REGISTRY  
FS PROTEIN SEQUENCE  
SQL 118

SEQ 1 FCIPT EYTMV VDRRE CAYCL TINTTICAGY CMTRDINGKL FLPKYALSQD  
51 VCTYRDFIYR TVEIPGCPHH VTPYFSFPVA VSCKCGKCNT DNSDCIHEAV  
=====

101 RTNYCTKPQS FYLGGSFV

HITS AT: 51-80

=> fil capl; s 16 or 17 or 110

FILE 'CAPLUS' ENTERED AT 15:10:18 ON 14 JUL 2000  
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FILE COVERS 1967 - 14 Jul 2000 VOL 133 ISS 3  
FILE LAST UPDATED: 13 Jul 2000 (20000713/ED)

Searched by Barb O'Bryen, STIC 308-4291

This file contains CAS Registry Numbers for easy and accurate substance identification.

This file supports REGISTRY for direct browsing and searching of all substance data from the REGISTRY file. Enter HELP FIRST for more information.

Now you can extend your author, patent assignee, patent information, and title searches back to 1907. The records from 1907-1966 now have this searchable data in CAOLD. You now have electronic access to all of CA: 1907 to 1966 in CAOLD and 1967 to the present in CAPLUS on STN.

1 L6  
1 L7  
4 L10  
L14 4 L6 OR L7 OR L10

=> d ibib abs hitrn l14 1-4; fil hom

L14 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1999:223048 CAPLUS

DOCUMENT NUMBER: 130:247459

TITLE: Mutants of thyroid stimulating hormone subunits with improved bioactivity and stability

INVENTOR(S): Weintraub, Bruce D.; Szkudlinski, Mariusz W.

PATENT ASSIGNEE(S): University of Maryland, Baltimore, USA

SOURCE: PCT Int. Appl., 44 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9915665	A2	19990401	WO 1998-US19772	19980922
WO 9915665	A3	19990520		
W:	AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, VZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
AU 9894998	A1	19990412	AU 1998-94998	19980922
EP 1017817	A2	20000712	EP 1998-948422	19980922
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI			
WO 2000017360	A1	20000330	WO 1999-US5908	19990319
W:	AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, VZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
AU 9931906	A1	20000410	AU 1999-31906	19990319
		Searched by Barb O'Bryen, STIC 308-4291		

## PRIORITY APPLN. INFO.:

US 1997-939472 19970922  
WO 1998-US19772 19980922  
WO 1999-US5908 19990319

AB The present invention is based upon the discovery that mutant .alpha. subunits and mutant .beta. subunits each comprising amino acid substitutions relative to the wild type can be produced and assembled to form a mutant TSH heterodimer or TSH analog that possesses higher bioactivity in vitro and longer half life in vivo. A preferred mutant .alpha. subunit (to be used in conjunction with a modification to increase the serum half-life of the TSH heterodimer having the mutant .alpha. subunit) comprises four mutations: Q13K, E14K, P16K, and Q20K; a preferred mutant .beta. subunit comprises three mutations: I58R, E63R, and L69R. Multiple mutations within a subunit and modifications to increase the half-life of the TSH heterodimer (i.e., .beta.-subunit fusion with the C-terminal extension peptide of human chorionic gonadotropin and/or a .beta. subunit-.alpha. subunit fusion) can act synergistically to achieve bioactivity that is greater than the sum of the increase of the mutations and the long acting modifications. Accordingly, the present invention provides methods for using mutant TSH heterodimers, TSH analogs, fragments, and derivs. thereof for treating or preventing diseases of the thyroid, in particular thyroid cancer. The invention also relates to methods of diagnosis, prognosis and monitoring for thyroid-related functions. Pharmaceutical and diagnostic comps., methods of using mutant TSH heterodimers and TSH analogs with utility for treatment and prevention of metabolic and reproductive diseases are also provided.

## IT 221650-43-7P 221650-47-1P

RL: BAC (Biological activity or effector, except adverse); BPN  
(Biosynthetic preparation); PRP (Properties); BIOL (Biological study);  
PREP (Preparation)  
(mutants of human TSH subunits with improved bioactivity and stability)

L14 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1988:417963 CAPLUS  
DOCUMENT NUMBER: 109:17963  
TITLE: Organization and nucleotide sequence of the gene  
encoding the .beta.-subunit of murine thyrotropin  
AUTHOR(S): Gordon, David F.; Wood, William M.; Ridgway, E.  
Chester  
CORPORATE SOURCE: Health Sci. Cent., Univ. Colorado, Denver, CO, 80262,  
USA  
SOURCE: DNA (1988), 7(1), 17-26  
CODEN: DNAADR; ISSN: 0198-0238  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB A murine genomic DNA library was constructed in .lambda. EMBL3 and the nucleotide sequence of the murine TSH .beta.-subunit (TSH.beta.) gene was detd. The cloned gene was derived from a thyrotropic tumor and had no detectable rearrangements when compared to the murine TSH.beta. gene in total genomic DNA. The murine TSH.beta. gene is 5 kb in size and consists of five exons and four introns. The 5' untranslated region of the mRNA is encoded except for a single nucleotide by exons 1, 2, and 3. The protein-coding regions are encoded by exons 4 and 5 while the 3' untranslated region is entirely contained in exon 5. Primer extension anal. using an exon 1-specific primer was used to map the 5' end of the gene. Two transcriptional start sites are present in the murine TSH.beta. gene which appear to be positioned by two TATAAA sequences located 40 bp apart. In all, 99% of transcripts initiate at the downstream site. Transcription from both start sites is affected by thyroidal status in both murine pituitaries and in TtT97 thyrotropic tumors. Finally, sequences homologous with putative-responsive elements and cAMP-responsive elements are present in the 5'-flanking region and may be important in regulating neg. and pos. effects on TSH.beta. gene expression.  
Searched by Barb O'Bryen, STIC 308-4291

IT 86179-23-9, Thyrotropin (mouse .beta.-subunit protein moiety reduced) 86179-24-0  
RL: PRP (Properties)  
(amino acid sequence of)

L14 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1988:50315 CAPLUS

DOCUMENT NUMBER: 108:50315

TITLE: Expression of the gene for the .beta. subunit of mouse thyrotropin results in multiple mRNAs differing in their 5'-untranslated regions

AUTHOR(S): Wolf, Ofra; Kourides, Ione A.; Gurr, James A.

CORPORATE SOURCE: Lab. Mol. Endocrinol., Meml. Sloan-Kettering Cancer Cent., New York, NY, 10021, USA

SOURCE: J. Biol. Chem. (1987), 262(34), 16596-603

CODEN: JBCHA3; ISSN: 0021-9258

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The gene encoding the .beta. subunit of mouse thyrotropin (TSH.beta.) was isolated from a mouse genomic library, and its nucleotide sequence has been detd. Blot hybridization anal. of restriction enzyme digests of mouse DNA indicates that there is a single mouse TSH.beta. gene. The gene is 4.8 kilobases in length and contains 5 exons, which are 27, 47, 41, 163, and 328 base pairs long. Exons 1, 2, and 3 encode only 5'-untranslated mRNA sequences and are sepd. by introns that are 150 and 380 base pairs long. The protein-coding mRNA sequences are found in exons 4 and 5 and are interrupted by a 460-base-pair intron. The position of this intron, between the codons for amino acids 34 and 35, has been conserved in all the known glycoprotein hormone .beta.-subunit genes. Exons 3 and 4 are sepd. by a large 3.2-kilobase intron. When primer extension anal., using an oligonucleotide primer complementary to exon 4 sequences, was employed to locate the transcription start site, 4 products were obtained. Nucleotide sequencing of these products showed that they were derived from sep. TSH.beta. mRNAs that differed in the lengths of their 5'-untranslated regions. These 5'-untranslated mRNA sequences are derived from different combinations of exons 1, 2, and 3, each spliced to exons 4 and 5. The longest 5'-untranslated sequence, 116 nucleotides long, includes exons 1, 2, and 3 and the first base of exon 4; the shorter 5'-untranslated regions, 75, 69, and 28 nucleotides long, arise by splicing out the second and(or) the third exon sequences. In contrast to the mouse TSH.beta. gene, transcription of the rat TSH.beta. gene from the analogous start site has been reported to give only a single mRNA, with a 5'-untranslated region of 28 nucleotides. Divergence of the mouse and rat TSH.beta. gene sequences at RNA splice sites can account for the absence of exon 2, but not exon 3, sequences in rat TSH.beta. mRNA. Primer extension and RNase protection analyses also showed that the mouse TSH.beta. gene contains a second transcription start site, located 43 base pairs upstream of the first start site, in a position corresponding to that in the rat TSH.beta. gene. Each start site in the mouse gene is flanked by characteristic TATAA box and CAAT box sequences. In the hypothyroid mouse pituitary and in mouse thyrotropic tumors, transcription occurs predominantly from the downstream start site. Elucidation of the structure of the mouse TSH.beta. gene and its 5'-flanking region facilitates the study of the mechanism controlling the prodn. of multiple mouse TSH.beta. mRNAs and the use of the alternative promoters.

IT 86179-23-9 86179-24-0  
RL: PRP (Properties)  
(amino acid sequence of)

L14 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1983:417486 CAPLUS

DOCUMENT NUMBER: 99:17486

Searched by Barb O'Bryen, STIC 308-4291

TITLE: Cloning of cDNA encoding the pre-.beta. subunit of mouse thyrotropin  
AUTHOR(S): Gurr, James A.; Catterall, James F.; Kourides, Ione A.  
CORPORATE SOURCE: Lab. Mol. Endocrinol., Mem. Sloan-Kettering Cancer Cent., New York, NY, 10021, USA  
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1983), 80(8), 2122-6  
CODEN: PNASA6; ISSN: 0027-8424  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Double-stranded cDNA was synthesized from sucrose gradient-purified poly(A)-contg. mRNA from a mouse thyrotropic tumor, inserted into the PstI site of plasmid pBR322 by using poly(dC).cntdot.poly(dG) homopolymeric extensions, and cloned in Escherichia coli RRI. Plasmids contg. cDNA sequences coding for the .beta. subunit of TSH [9002-71-5] were identified by cell-free translation of hybrid-selected mRNA and immunopptn. with specific antibody to TSH .beta. subunit. Detn. of the nucleotide sequence of 1 cDNA, 595 base pairs in length, allowed deduction of the entire amino acid sequence of the mouse TSH .beta. subunit. The pre-.beta. subunit contains a 20-amino acid N-terminal signal sequence followed by a 118-amino acid mature TSH .beta. subunit. There is 85-90% homol. in amino acid sequence between mouse TSH .beta. subunit and subunits from man, pig, and cow; however, the mouse subunit contains an addnl. 5 and 6 amino acids at its C-terminus compared to the bovine or human and pig subunits, resp. TSH .beta.-subunit mRNA from a mouse thyrotropic tumor was estd. to be 750 nucleotides in length by hybridization with labeled TSH .beta.-subunit cDNA.

IT 86179-23-9 86179-24-0  
RL: PRP (Properties)  
(amino acid sequence of)

FILE 'HOME' ENTERED AT 15:10:32 ON 14 JUL 2000

## Sequence Family Search of Proteins (/sqsf)

In the sequence family search, each amino acid in the query has to match either the exact amino acid or a family member equivalent, as shown in the Family Equivalence Table below. The Family Equivalence Table is applied only to each common amino acid in the sequence. Specific uncommon amino acids may be included in the sequence; however, family equivalents only exist for the common amino acids. An amino acid family is based on a conservative substitution of amino acids sharing a similar chemical property. Each common amino acid in the query is converted to its family class members in a search. A match occurs on a query sequence if each amino acid is exactly matched or any of its family members are encountered. For example, the Hydrophobic-Aromatic family consists of the common amino acids F, W, and Y. If the amino acid F is specified within a sequence exact family search, it will match on amino acids F, W, or Y.

### FAMILY EQUIVALENCE TABLE

Family Class Name	Family Class Members
Neutral-Weakly Hydrophobic	Ala (A), Gly (G), Pro (P), Ser (S), Thr (T)
Hydrophilic-Acid Amine	Asn (N), Asp (D), Gln (Q), Glu (E)
Hydrophilic-Basic	Arg (R), His (H), Lys (K)
Hydrophobic	Ile (I), Met (M), Leu (L), Val (V)
Hydrophobic-Aromatic	Phe (F), Trp (W), Tyr (Y)
Crosslinking	Cys (C)